Comment Sheet

APPLICATION SERIAL NUMBER

S/N 10/556,069

DOES NOT COMPLY WITH THE SEQUENCE RULES. See reasons below.

On page 17 several specific polynucleotide sequences greater than 10 bases are set forth and require a SEQ ID NO. (see attached sheet of spec).

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merase, 200µM dNTP each, 25pM of each primer and 0.2µg of plasmid DNA as template). The extension time (72°C) differed for each construct and is described separately in the following:

Construction of plasmid pSIPI

To place the resolution sites at the desired positions in the SIP vector it was necessary to introduce suitable restriction sites in plasmid pSIP to enable directional cloning of the resolution sites. Plasmid pSIP was linearised with HindIII and subsequently digested with NaeI. The resulting 2995 bp HindIII / NaeI fragment was eluted from an agarose gel. This fragment contains the regulatory elements of the araBAD operon and the essential elements for self immobilisation. A 3131 bp-DNA fragment containing the β-lactamase gene and origins of replication of M13 and of pBR322 was generated by PCR-amplification (extension time: 7 min) using the primers pSIPI5 (5′-CAGCAGAAGCTTGTTTTG-GCGG- ATGAGAGAAG-3′) and pSIPI3 (5′-AGATCTCTGCTGGCGGCCGCGGTTGCT-GGC- GCCTATATC-3′) and the vector pSIP as template. Primer pSIPI5 contains a single HindIII site, pSIPI3 contains a BglII and a NotI restriction site. After digestion of the PCR-fragment with HindIII it was ligated with the 2995bp fragment described above via their blunt site obtained by the Pfu polymerase and the corresponding HindIII site, respectively, resulting in the vector pSIPI. Due to the cloning strategy following restriction sites were introduced: SacII, NotI, BglII, BalI.

Construction of plasmid pSIPIres

The resolution sites were generated by PCR amplification (extension time: 1 min) using the vector pJMS11 as template and the following primer sets: resolution site 1: 5res1: 5 ′-CAGCAGCTGCAGCCTTGGTCAAA-TTGGGTATACC- 3′; 3res1: 5′-CTGCT-GAAGCTTGCACATATGTGGGCGTGAG-3′; resolution site 2: 5res2: 5 ′-CAGCAGGCGGCCGCCCTTGGTCAAATTGGGTATACC-3′ 3res2: 5′-CTGCTGAG-ATCTGCACATATGTGG GCGTGAG-3′. The PCR-fragment encoding the resolution site 2 contained a unique NotIsite at the 5′end and a BglII site at the 3′end and was cloned into the corresponding single sites of pSIPI resulting in the vector pSIPIres2. The resolution site 1 fragment containing a single PstI site at the 5′end and HindIII site at the 3′end, respectively, was then cloned into the corresponding single sites of pSIPIres2 resulting in the vector pSIPIres.

Construction of plasmid pSIPIresHCN